Indigenous lactic acid bacteria population found in Rioja Alavesa red wines fermentations and their ability to produce biogenic amines and ethyl carbamate

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E-mail: idiez@azti.es

I. Diez-Ozaeta[1]., F. Amárita[1] ., F. J. Pérez-Elortondo[2, 3]., S. Rainieri[1]

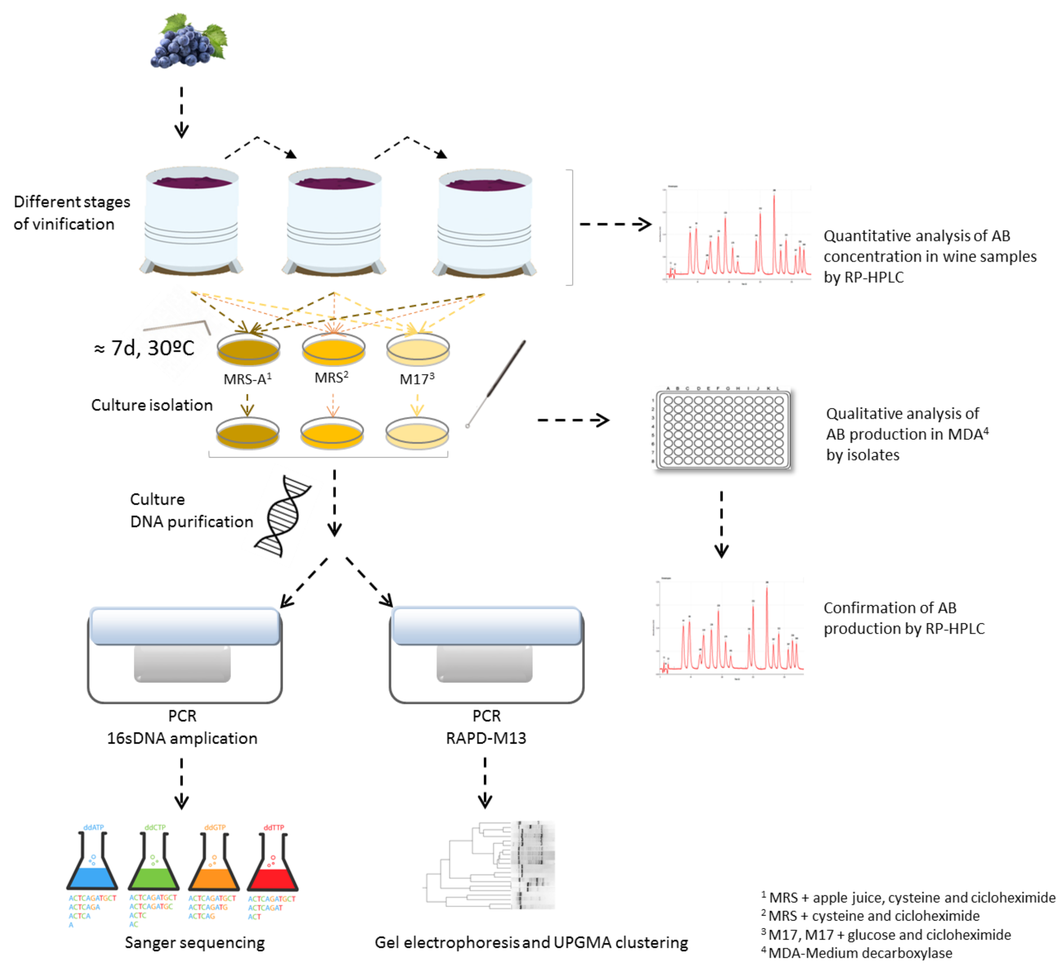
INTRODUCTION

The employment of indigenous strains as starters for wine fermentations has become a common practice to improve **malolactic fermentation (MLF) efficiency and reliability**. Microorganisms presenting resistance to the stressful environment found in wine and the ability to produce desirable sensory compounds are of great value in winemaking industry.

In addition, the development of high quality wines requires the absence of metabolites of safety concern such as **biogenic amines (BAs)** and **ethyl carbamate (EC)** which are generally promoted by microorganisms naturally present in the enological environment.

The **objective** of the present study was to identify interesting **indigenous lactic acid bacteria (LAB) not producing metabolites of safety concern and suitable to be used as starter cultures.** To that end 33 samples of must and wine at all stages of vinification were analyzed from different wineries of the Rioja Alavesa region, a portion of the Qualified Denomination Of Origin Rioja, in the north of Spain.

MATERIALS AND METHODS



[1] MRS + apple juice, cysteine and cicloheximide
[2] MRS + cysteine and cicloheximide
[3] M17, M17 + glucose and cicloheximide
[4] MDA-Medium decarboxylase

RESULTS AND DISCUSSION

> Typing of isolates by RAPD-PCR

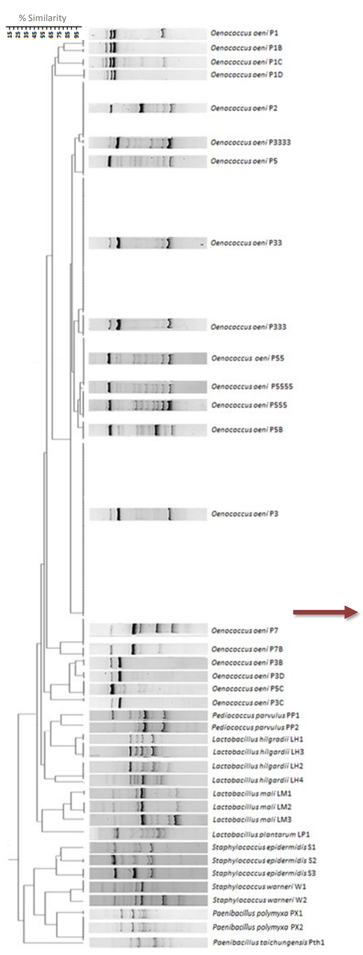


Figure 2: Dendrogram derived from clustering analysis of M13 RAPD-PCR patterns of all isolates. Clustering analysis was carried out using the Unweighted Pair Group Method with Arithmetic Average (UPGMA)

> Bacterial population found during winemaking process

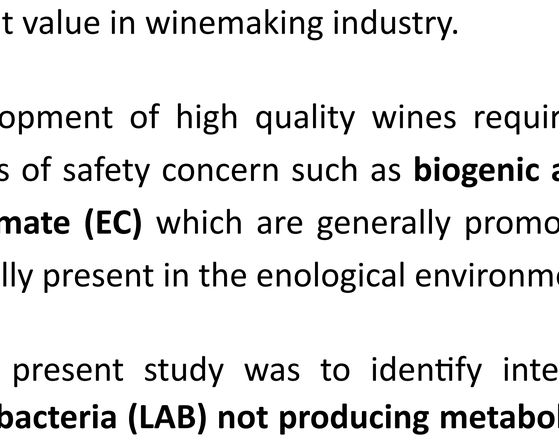


Figure 1: Percentage of bacteria isolated at different stages of winemaking process (1: must; 2: tumultuous MLF; 3: final MLF)

/ *O.oeni*, main species (>98% final MLF)
/ Uncommon genus: *Staphylococcus* and *Paenibacillus*

> Analysis of *O. oeni* strains diversity

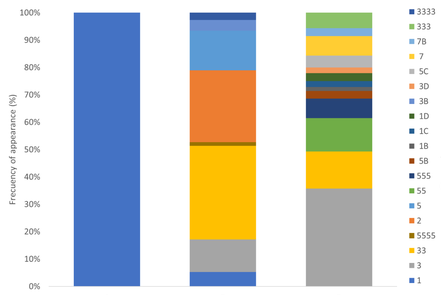


Figure 3: Frecuency of appearance (%) of *O. oeni* genotypes at different stages of winemaking process (1: must; 2: tumultuous MLF; 3: final MLF)

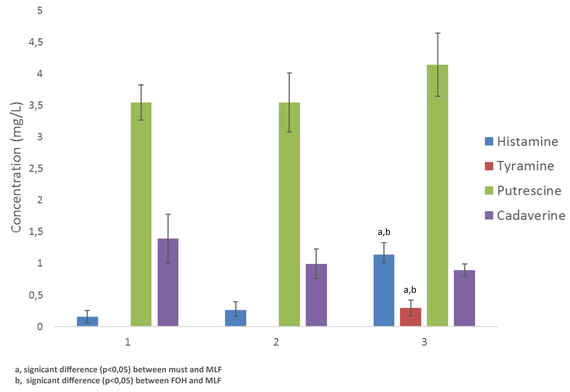
Table 1: Number of isolates and genotypes of *O. oeni* during must (1), tumultuous MLF (2) and final MLF (3)

Stages	1	2	3
Nº of total isolates	13	118	143
Nº *O.oeni* isolates	2	76	142
Nº *O.oeni* genotypes	1	8	14
ID*	-	0.78	0.83

*ID, Simpson´s diversity index.
(-), incalculable.

/ Low species diversity/High genotypic diversity
/ *O. oeni* genotypic richness during MLF

> Quantitative analysis of biogenic amines during winemaking process by RP-HPLC



a, signicant difference (p<0,05) between must and MLF
b, signicant difference (p<0,05) between FOH and MLF

Figure 4: Quantification by RP-HPLC of histamine, tyramine, putrescine and cadaverine of samples at different stages (1: must; 2: FOH; 3: MLF) from which current bacterial isolates were obtained.

/ Significant increase in histamine and tyramine at the end of MFL

> Ability of isolated bacteria to produce biogenic amines and degrade arginine

Table 2: Detection of AB production by phenotypical analysis in MDA media and subsequent RP-HPLC confirmation. In addition, arginine degradation pathway was evaluated, an enzyme kit (Megazyme ™) was used for NH_3 quantification and RP-HPLC for putrescine.

		Histamine		Tyramine		Putrescine (via agmatine)		Putrescine (via ornithine)		Cadaverine		Arginine	
	Nº isolates	MDA*	HPLC**	MDA	HPLC	MDA	HPLC	MDA	HPLC	MDA	HPLC	NH_3	Putrescine
Oenococcus oeni	234	7	n.d.	37	n.d.	2	3 (13,92-517,15)	1	n.d.	1	n.d.	**93 (39,96-4589,98)**	-
Lactobacillus hilgardii	18	-	-	-	-	-	-	1	n.d.	1	n.d.	**16 (67,01-1071,01)**	-
Lactobacillus mali	7	-	-	3	n.d.	1	1 (16,06)	1	n.d.	-	-	1 (153,6)	-
Pediococcus parvulus	2	-	-	-	-	-	1 (21,51)	-	-	-	-	1 (67,26)	-
Lactobacillus plantarum	1	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus pasteuri	7	2	1 (113,24)***	2	n.d.	2	2 (24,73-85,65)	2	2 (52,79-7693,13)	2	1 (4918,61)	2 (718,14-1150,87)	n.d.
Staphylococcus epidermidis	7	7	3 (608-1670)	6	1 (22,03)	6	5 (16,01-1227,66)	6	5 (217,39-7201,75)	5	3 (189,97-407,97)	7 (526,56-1333,71)	3 (349,70-1213.18)
Paenibacillus polymyxa	11	7	1 (173,30)	8	n.d.	6	6 (20,51-328.72)	6	7 (53,43-7922,52)	6	6 (11,71-185,05)	9 (72,24-1254,38)	2 (105,28-811.17)
Paenibacillus taichungensis	1	1	n.d.	1	n.d.	1	1 (370,09)	1	1 (150,72)	1	n.d.	1 (959,14)	n.d.
Bacillus subtilis	1	-	-	-	-	-	-	-	-	-	-	-	-

*MDA, number of positive isolates in decarboxylase media
**HPLC, number of positive isolates by HPLC
()***, concentration in mg/l
n.d., not detected

/ Low incidence of AB production by LAB
/ High aminobiogenic capacity of *Staphylococcus* and *Paenibacillus*
/ High rate of arginine degrading *O. oeni* via ADI pathway

CONCLUSION

Oenococcus oeni was the most abundant species found in this study. It showed a great genotypic diversity underlying its major role in MLF. Although species diversity was moderate, species rarely found in wine such as *Staphylococcus* and *Paenibacillus* which exhibit great aminobiogenic capacity were detected. LAB ability to produce AB was minor; however, it was detected a great number of strains belonging to *O. oeni* and *L. hilgardii* with a high capacity of degrading arginine via ADI pathway, highlighting the potential risk for the formation of EC precursors.

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[1]AZTI-Tecnalia, Food Research Division, Derio, Spain
[2]LASEHU, [3]Lactiker (University of the Basque Country-UPV/EHU). Vitoria-Gasteiz, Spain

EUSKO JAURLARITZA GOBIERNO VASCO